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Utilisation Gold Snail Flour Fermented *Papain Enzyme* in Feed as *Biofermentor* to Increase Growth of *Channa Striata*

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Abstract*— This study aimed to measure the optimal concentration of the enzyme papain that can be used in the snail goldfish fermentation to improve the performance of *Channa Striata*. This research conducted in May to September 2019 In Freshwater Fish Seed Center (BBI) Limbung, Gowa Regency. The feed used is pellet feed made from the gold snail, which segmented with the papain enzyme papaya. This study uses a Completely Randomized Design (CRD) consisting of four treatments and three replications, namely: A 1.5%, B 2.25%, C.3% and D. Control (0%). The data obtained were processed using Analysis of Variance (ANOVA) Duncan. The results showed the feeding of golden snails fermented by the enzyme papain with different concentrations provided (P <0.05) the level of protein digestibility, fiber digestibility, protein retention, feed efficiency, albumin reserves, and calcium growth. However, no significant effect (P> 0.05) on fat retention and *Channa Striata* fish seed survival. Protein digestibility, fiber digestibility, protein retention, feed use efficiency, albumin content, and balance growth. The highest value at the time of preparation B (2.25%) with the respective values (87.66%), (91.24%), (58.07%) (79.05%), (9.77%), (94gr) while the level of protein digestibility, fiber digestibility, protein retention, feed efficiency, albumin content, and concentration growth. The lowest obtained in treatment D (0%), were (60.09%), (81.72%), (32.56%), (54%), (7.88%) and (89.11gr). Water quality in the maintenance media during the study was 26-290C, pH 7-8, Oxygen 4-6mg / l and ammonia 0.013-0.11 mg / l.

***Keywords*—papain enzyme; growth performance; *Channa Striata*.**

I. INTRODUCTION

Channa Striata fish is a type of freshwater carnivorous fish that is commonly found in Southeast Asia. However, little is known about its history and biological nature. This type of fish is known as a fish that is widely consumed and sold on the market. In small size, *Channa Striata* fish look exotic so that many are used as ornamental fish in aquariums. In Indonesia, this fish is known by many regional names. Malaysians, Banjarmasin and Banjarnegara call it *Aruan* or *Haruan*. Betawi people call it *Kocolan*. The Sidiarjo people call it *Bogo*. Banyumas people call it *Bayong* or *Licingan*. Javanese call it a *Kutuk*. In English, Channa Striata fish is known by several variants of names, including the common snakehead, chevron snakehead, and stripped snakehead. The Sundanese call it *Rajong*. The Acehnese call it *Bace*. Palembang people call it *Sepunkat*.

In general, Channa Striata fish body is generally brown. The upper part is mostly black and the abdomen is light whitish. *Channa Striata* fish head is rather flat and shaped like a snake with large scales above the head, therefore *Channa Striata* fish are also dubbed as "snake head". The upper side of the *Channa Striata* fish body from head to tail is dark, brownish black or greenish. The underside of the body is white from the chin to the back. The sides are thick and slightly blurred. The color adjusts to the habitat environment. The mouth of a large *Channa Striata* fish with sharp teeth. The dorsal fin extends with a rounded caudal fin at the end.

One solution to get alternative and quality fish feed to encourage increased production of fish farming business is to use golden snails as feed. Snail meat can be given to fish in a raw (fresh) or processed form. In the development of fish farming, golden snail is a mixed feed as a cheap source of protein. Besides containing a lot of protein, golden snails are also rich in calcium.

Channa striata are one of the leading commodities of freshwater fish that is easily cultivated and has high economic value because it contains albumin. However, the problem is the *Channa Striata* fish is very dependent on the supply and suitability of the nutritional content of the feed, to produce optimal growth *Channa Striata* fish need 50% protein [1], [2]. The price of feed with high protein content is relatively expensive because protein sources such as fish meal and soy flour still imported from other countries [3] . For this reason, feed engineering efforts have been carried out, including by substituting a fish meal and soybean flour with golden snail flour [1], [4]. But the next factual obstacle faced is the low digestibility value of fish compared to the use of the fish meal [2]. So that the impact on moderate growth. For this reason, it is necessary to ferment the golden snail so that the protein contained in it can be entirely decomposed into amino acids, and one of the proteolytic enzymes that can break the peptide bonds in the protein is the papain enzyme [5].

Making golden snail shell, processing shell into flour is the same as golden snail meat flour i.e. do not feed the snail for two days, then separate it from the meat [6], [7]. Next the golden snail shell is cleaned of dirt and dried. Mash the shells in one container and grind it with a grinding machine. Meat flour and golden snail shells are ready to be mixed in animal feed as nutritional enhancers. Making golden snail silage, clean and remove the fresh snail meat from its shell. Wash the meat that has been removed thoroughly, with plain water. Wash again with salt water and wash it again with lime water, so that the animal feed produced is protected from poison. The meat is then ground with a grinding machine and then drained. Mix the ground conch meat with bran, in a ratio of 4: 1. Prepare a plastic barrel, then fill it with a mixture of conch and rice bran that had been prepared. Compact until there is no air cavity and cover with plastic tightly. The process of making animal feed in the form of snail silage takes 12 days for perfect fermentation. After that silage can be used for animal feed.

The protein content in the golden snail silage is very useful as an additional feed and can help accelerate the growth of livestock [8]. The benefits of making snail silage for animal feed in addition to preserving snail meat, also to activate cellulose substances through the fermentation process. With active ingredients can facilitate livestock in digesting food and can shorten the absorption of nutrients. Feed is one of the factors that can support the development of fish farming. The food consumed by fish in aquaculture activities must contain nutrients that are easily digested and well absorbed by cultivation, so that the feed can be used optimally for growth. In the manufacture of artificial feed can also be applied to the papain enzyme, the addition of the papain enzyme can provide added value to the quality of artificial feed. The papain enzyme is a protease enzyme derived from papaya plants (*Carica papaya*). The addition of the enzyme papain into artificial feed, will be able to accelerate the growth of cultivation (cultivation organisms).

Research on the use of the enzyme papain as a fish food supplement has been carried out, among others [1], [5], [9] However, research has not been carried out on the substrate of golden snail mashed by the enzyme papain in feed to improve the growth performance of *Channa Striata* fish. This study aims to determine the optimal levels of the enzyme papain used for the process of golden snail fermentation as a raw material for fish feed on the growth performance of *Channa Striata* fish.

II. MATERIAL AND METHOD

Test fish used was *Channa Striata* fish seed weight ± 1 g. *Channa Striata* fish that are sampled first are acclimatized to the maintenance environment for 1 hour then feed adaptation is carried out for one week before being given test feed according to treatment. The test feed used in this study was pellet feed whose raw material was fermented papain snail mas. The process of making feed starts with the preparation of raw materials, fermentation, mixing of feed raw materials, molding of feed and drying of feed. Feeding is carried out at satiation three times per day. *Channa Striata* fish are kept for two months to reach the objective of the research. To get proximate quantitative data did by spectrophotometer. The steps in determining the body's proximate follow the instructions from [10]. Initial and final observations were made to obtain growth fish data. The containers used are aquariums with length, width and height of 50 x 40 x 35 cm each. The sides of the container are covered with black plastic, as shown in Figure 1.

Fig. 1. Research Containers

A. Experiment Design

The research applied a Completely Randomized Design (CRD) consisting of four treatments and three replications. The treatment is as follows:

Treatment A: Papain enzyme 1.5% / kg golden snail
Treatment B: Papain enzyme 2.25% / kg golden snail
Treatment B: Papain enzyme 3.00% / kg golden snail
Treatment D: Control

B. Observed variables

1) Nutrient Digestion (Protein Digestion and Feed Fiber Digestion: The digestibility value of protein/feed fiber is calculated based on the equation [11] below.

$$\text{Fiber digestibility} = (\mathbf{1} + \frac{\boldsymbol{a}}{\boldsymbol{a'}} \boldsymbol{x} \frac{\boldsymbol{b'}}{\boldsymbol{b}}) \text{x } 100\% \quad (1)$$

Information:

a = % Cr2O3 in feed
a' = % Cr2O3 in faces
b = % fiber in feed
b' = % of the fiber in the stool

2) Protein Retention and Fat Retention: to determine the increase in protein, fat and energy, a proximate analysis is performed. The investigation was conducted at the beginning and end of the study [10]. The percentage of nutrient retention is calculated using the formula below [11].

$$RP = \frac{(F_p - I_p)}{P} x100\% \quad (2)$$

Information:

Fp: the amount of fish body protein at the end of the study (g)
Ip: Amount of fish body protein at the beginning of the study (g)
P: Amount of Protein consumed by fish during the study (g)

3) The Efficiency of Feed Utilization (EPP): The efficiency of feed utilization is calculated using the formula below [12].

$$EPP = \frac{W_t - W_o}{F} x100\% \quad (3)$$

Information:

EPP: Feed utilization efficiency (%)
Wt: Biomass of fish at the end of the study (g)
Wo: Biomass of fish at the beginning of the study (g)
F: Total weight of the feed during the study (g)

4) Measurement of albumin levels: Measurement of albumin levels was carried out at the beginning and end of the study using fillet fish; then the albumin content calculated using a spectrophotometer.

5) .Growth is calculated using the absolute growth formula [11] *as follows:*

$$W = W_t - W_0 \quad (4)$$

Weight measurements were performed at the beginning of the study, mid and end of the study. The measured weight is the weight of the biomass which is then averaged for each fish

6) Survival Rate

Seed survival is calculated by the formula [13]

$$SR = \frac{N_t}{N_0} \text{ x } 100\% \quad (5)$$

Information:

SR: Survival (%)
N_t: Number of fish at the end of the study (tail)
N_0: Number of fish at the beginning of the study (tail)

Water quality is measured using tools such as thermometers used for temperature measurement, pH meters used in measuring pH (acidity) on maintenance media, DO meters used in DO measurements (dissolved oxygen), and Water Quality Checker used for measurement of NH3 (ammonia) content in the treatment media.

C. Data analysis

Protein digestibility, fiber digestibility, protein retention, fat retention, feed utilization efficiency, albumin levels, growth and survival in each treatment were analyzed using variance (ANOVA) if there was a significant effect then continued with Duncan's continued test 95% confidence interval (95% confidence interval)) used SPSS version 24. The water quality analyzed descriptively.

III. Result and Discussion

A. Results

1) Digestion of Channa Striata Fish Seed Nutrients:

The study presented average values of protein digestibility and digestibility of *Channa Striata* fish seed fiber-fed with fermented papain enzymes with different concentrations showing in Table 1.

TABLE I
DIGESTION LEVEL OF NUTRIENT FEED OF *CHANNA STRIATA* FISH SEEDS FED WITH SNAILS FED FERMENTED PAPAIN ENZYMES WITH DIFFERENT CONCENTRATIONS

Enzyme Concentration Treatment (%)	Retention	
	Protein Retention	Fat Retention
Treatment A (1,5 % papain enzyme)	51.07±0.15^{b}	3.04±0.19^{a}
Treatment B (2.25% papain enzyme)	58.07±0.43^{a}	13.08±0.77^{a}
Treatment C (3% papain enzyme)	48.04±0.51^{b}	13.12±0.95^{a}
Treatment D (control)	32.56±0.91^{c}	13.16±0.11^{a}

Note: Average values in the same column with different superscript letters indicate significantly different values

Based on the analysis of various types of substitution of golden snails fermented by the enzyme papain with different doses, it gave a significant effect ($P < 0.05$) on the digestibility of feed nutrients of *Channa striata* seeds. The results of Duncan's further test of providing treatment of golden snail fermented papain enzymes with different doses showed that the digestibility of feed protein of *Channa Striata* fish seed substituted by golden snail fermented papain enzyme at a dose of 2.25% was very significant ($P < 0.05$) higher than the substitution of fermented golden snail 1.75 %, 3% and 0%.

The results in Table 1 showed that the feed made from gold snail fermented by the enzyme papain 2.25% produces the highest nutrient digestibility value compared to the administration of other enzyme concentrations. It is because the enzyme concentration of 2.25% is the optimal and effective concentration for *Channa Striata* fish seeds. Enzymes function for the complete breakdown of protein-peptide bonds into more straightforward peptide bonds [14]. Feed provided can be digested by *Channa Striata* fish more perfectly. The low value of protein digestibility in the 3% papain enzyme fermentation treatment showed *Channa Striata* fish seeds did not effectively utilize the concentration in breaking down protein into amino acids, increasing the dose of the papain enzyme is able to damage the protein balance or configuration so that the feed given cannot be digested optimally by fish [15]. Likewise, at concentrations of 1.5% and 0% (control), the level of the enzyme is not effective in breaking down proteins into simpler forms. It is following the results of [3], [16], [17] that the enzyme concentration of 2.25% is the optimal concentration for *Sangkuriang* catfish seeds in hydrolyzing the feed protein given compared to papain concentrations (1.125% / kg of feed) and (3.375% / kg of feed).

2) Protein Retention and Fat Retention

The average values of protein retention and fat retention of *Channa Striata* fish seeds were fed with fermented papain enzymes with different concentrations presented in Table 2.

Based on the analysis of various types of substitution of golden snails fermented by the enzyme papain with different doses gave a significant effect (P <0.05) on protein retention, but did not significantly affect the retention of *Channa Striata* fish seed fat. Duncan further test results showed that the retention of *Channa Striata* fish seed protein substituted by golden snail fermented by the enzyme papain at a dose of 2.25% was very significant (P <0.05) higher (58.07) than the substitution of fermented golden snails 1.75%, 3% and 0 %.

TABLE II
THE AVERAGE VALUE OF PROTEIN RETENTION AND FAT RETENTION OF *CHANNA STRIATA* FISH SEEDS FED WITH GOLDEN SNAIL FERMENTED PAPAIN ENZYMES WITH DIFFERENT CONCENTRATIONS

Enzyme Concentration Treatment (%)	**Feed Nutrient Digestion**	
	Protein Digestion	**Fiber Digestion**
Treatment A (1.5 % papain)	76.34±0.15[b]	86.83±0.19[b]
Treatment B (2.25% papain)	87.66±0.43[a]	91.24±0.77[a]
Treatment C (3% papain)	75.32±0,51[b]	85,44±0,95[b]
Treatment D (control)	60.09±0.91[c]	81.72±0.11[c]

Note: Average values in the same column with different superscript letters indicate significantly different values.

The results of the study in Table 2 show that the golden snail's fermentation with an enzyme concentration of 2.25% produced the highest protein retention value of 58.07 ± 0.43a compared to other treatments. The high retention value of feed protein in the 2.25% enzyme fermentation treatment in this study is directly proportional to the percentage of protein digestibility. It is because effectively digested feed protein can utilize and absorbed by the *Channa Striata* fish's body completely, causing the body's protein content to be higher compared to other treatments. It paralleled results of research by [1] which produces a concentration of 2.25% which is the optimal concentration in producing protein retention. While the retention value of protein produced in this study was 58.07 ± 0.04 higher than the retention of protein produced by [1] in the research of feeding snails, feed on *Channa Striata* fish seeds which were not fermented with retention values of 49.13 ± 1.07.

The results of the study in (Table 2) showed the substitution of golden snails fermented by the enzyme papain with different doses did not have a significant effect (p> 0.05) on the retention of *Channa Striata* fish seed fat. It is because the papain enzyme does not work in the process of fat hydrolysis [5], other than that the fat content in the feed for all treatments in this study is the same. The research is line with [1], which obtained no different fat retention in *Channa Striata* fish fed chromium supplement which was incorporated through *Rhizopus orizae* mushroom with the same fat content but containing different protein and carbohydrate levels. It is also in line with the statement of [5], [9], [18] that papaya contains a shallow concentration of the lypase enzyme compared to other fruits such as avocados and bananas so that the enzyme activity is also superficial.

3) Feed Efficiency

The average value of feed efficiency of *Channa Striata* fish seeds fed with snails fed fermented papain enzymes with different concentrations during the study presented in Figure 2.

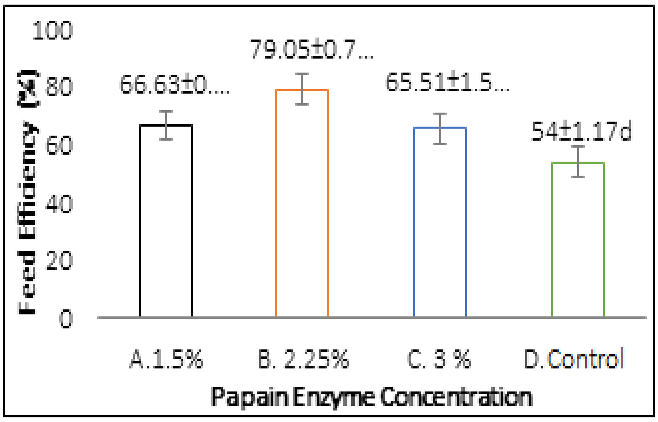

Fig. 2 The efficiency of *Channa Striata* fish seed feed fed by snails of fermented papain enzymes with different concentrations during the study

Analysis of variance showed that the administration of the golden snail fermented by the enzyme papain had a real effect (p <0.05) on the feed efficiency of *Channa Striata* fish seeds. Duncan further test results showed that the effectiveness of feed fish *Channa Striata* fish fed goldfish snail fermented papain enzyme 2.25% significantly higher than other test feeds.

Based on Figure 2. The highest value of feed efficiency produced in this study was obtained in treatment B 2.25% enzyme concentration, i.e. 79.05 ± 0.73 compared to treatments A, C and D (1.5%, 3% and 0%). It is because the golden snail fermentation with papain enzyme 2.25% is the optimal concentration that effectively hydrolyses protein into protein in a more straightforward form so that *Channa Striata* fish seeds can digest food optimally so that the feed given can utilize efficiently. The results of this study are under that reported by [19]–[21] that hydrolysis that occurs with proteolytic enzymes in the breakdown of peptide bonds from substrate bonds and protein hydrolysis is carried out by endogenous enzymes and assisted by exogenous enzymes where hydrolytic enzymes act as exogenous enzymes. The addition of this enzyme helps produce more amino acids so that the feed consumed can be utilized more efficiently [22], [23].

4) Growth

The average growth value of *Channa Striata* fish seeds fed with snails fed fermented papain enzymes with different concentrations is presented in Figure 3. Based on the analysis of various types of substitution of golden snails fermented by the enzyme papain with different doses gave a significant effect (P <0.05) on the growth of *Channa Striata* fish seeds. Duncan further test results showed that the growth of *Channa Striata* fish seeds substituted by golden snail fermented by the enzyme papain at a dose of 2.25% was very significant (P <0.05) higher (94.07) than the substitution of fermented golden snails 1.75%, 3% and 0 %.

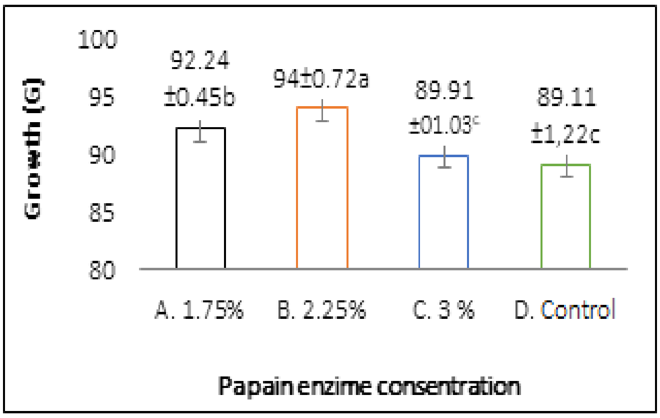

Fig. 3 Growth of *Channa Striata* fish seeds fed goldfish snail fermented by the enzyme papain with different concentrations in artificial feed during the study.

The high growth obtained in treatment B (2.25%) shows that the administration of papain enzymes at these concentrations can hydrolyze complex proteins into simpler proteins that produce more amino acids so that the food consumed can be utilized efficiently and the energy supply contained in the feed exceeds the energy requirements for maintenance and other bodily activities, so that the excess energy is then used for growth. It is in line with the results of research by [1], [4], [24] which states that before growth occurs, the energy needs for maintenance must be met first. Furthermore, the presence of enzymes in artificial feed can help and speed up the digestion process so that nutrients can be sufficiently available for growth [2], [24].

The low growth obtained in the treatment of 1.5% and 3% enzyme concentrations is in line with the results of research [25] that digestive viscosity will increase if the quantity of enzymes is too low. Digestion and absorption of feed nutrients will be hampered due to the digestive viscosity of non-starch polysaccharides derived from insoluble carbohydrates that result in weak growth [26]. While excess enzyme doses will result in excessive release of monosaccharides, resulting in hyperglycemia. Hyperglycemia can inhibit growth. According to [27], hyperglycemia is a condition of high sugar levels. Hyperglycemia can weaken insulin secretion and increase the weight of insulin retention. Based on the analysis of various types of substitution of golden snails fermented by the enzyme papain with different doses did not have a significant effect ($P < 0.05$) on the survival rate of *Channa Striata* fish seeds

5) *Water Quality Parameters*

The results of the water quality measurement of *Channa Striata* fish maintenance media during the study can be seen in Table 3.

TABLE III
WATER QUALITY PARAMETERS

Water Quality Parameters	The Range	Eligibility (Library)
Temperature	26 – 29°C	25 – 32°C (Boyd,1982)
Dissolved Oxygen	5 – 7,8 mg/L	> 3 mg/L (Zonneveld *et al.* (1991)
pH	7– 8	6,5 – 9,0 (Zonneveld *et al.* (1991)
Ammonia	0,013-0,11 mg/L	<1 mg/L (Robinette (1976)

The results of the measurement of water quality parameters indicate that the value of water quality parameters during the determination is still in the range that is suitable and feasible for *Channa striata* fish culture.

B. *Discussion*

Channa Striata is a type of freshwater fish with 30 species spread from Africa to Asia. In Asia, this species is spread from Afghanistan, western Pakistan, southern Nepal, India, Bangladesh, Sri Lanka, Myanmar, Indo-China, China, Japan, Taiwan, the Philippines, Malaysia, Singapore and western Indonesia. Southeast Asia is the center of the spread of cork fish with 10 species in it. As many as five species spread in Singapore, Malaysia and Indonesia, including *Channa micropeltes*, *Channa striata*, *Channa lucius*, *Channa melasoma*, and *Channa gachua*. Some species of Channa which are spread in Malaysia, Singapore, Sumatra, Kalimantan (Borneo) include *Channa sp.*, *Channa bankanensis*, *Channa gachua*, *Channa lucius*, *Channa marulioides*, *Channa melanoptera*, *Channa melasoma*, *Channa micropeltes*, *Channa pleurophthalma*, and *Channa striata* [28]–[32].

Channa Striata are generally found in shallow waters such as rivers and swamps with a depth of 40 cm and tend to choose a dark, muddy, calm current, or rocky area to hide. In addition, this species is also found in lakes and water channels to the rice fields. *Channa Striata* is one type of freshwater fish that has a wide spread, and naturally can live in lakes, rivers, freshwater swamps, and rice fields. *Channa Striata* seeds are found in many areas of grass or water plants and shrubs that are submerged in water.

In general, *Channa Striata* have an allometric growth pattern or gain weight faster than body length gain, this is related to its aggressive nature in foraging. These fish prey on a variety of small fish, insects, and various other aquatic animals including tadpoles and frogs. The natural food of *Channa Striata* is aquatic animals such as small fish, frogs and aquatic insects. *Channa Striata* has the ability to breathe directly from the air, using a kind of labyrinth organ called a diverticulum located at the top of the gills so it is able to breathe air from the atmosphere. Like fish that have a labyrinth, *Channa Striata* are able to survive in the conditions of marsh waters with low dissolved oxygen content and pH around 4.5,6. In the process of spawning this species has a habit of building a foamy nest among the vegetation in its environment. *Channa Striata* make foam nests around aquatic plants in swamps and shallow waters with weak currents. The foam is shaped like a circle that functions not only as a spawning area but also as a protective egg that has been fertilized.

By making their own feed can reduce the cost of feed can certainly increase profits that are more feasible for farmers. Making independent food as an effort to support the Gerpari (Independent Fish Feed Movement) program launched by the Ministry of Maritime Affairs and Fisheries, Indonesia. Independent feeds made in the application of the protease enzyme technology are artificial feed in the form of pellets. The addition of the enzyme papain can increase growth and efficiency of feed utilization.

The technology of adding papain protease in artificial feed can increase feed digestibility, feed absorption and feed utilization efficiency so that it can accelerate growth, reduce the amount of artificial feed given during aquaculture activities and reduce high production costs. The enlargement of environmental management is carried out by utilizing biotechnology products in the form of probiotics and system biofilter. With the application of probiotic technology there will be competition between pathogenic and non-pathogenic bacteria, so that specific diseases can be suppressed, and supported by the use of a biofilter system in catfish enlargement maintenance media, media water quality will always be maintained and ultimately will result in increased production in fish enlargement.

IV. Conclusions

The golden snail substitution which fermented with papain enzyme with a concentration of 2.25% produces protein digestibility, fiber digestibility, protein retention, feed efficiency, albumin and optimal growth in *Channa Striat*a fish seeds. Substitution of golden snails fermented by the enzyme papain with different concentrations does not affect the survival rate of *Channa Striata* fish seeds.

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